PARTICLE SIZING BY HYDRODYNAMIC CHROMATOGRAPHY

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PARTICLE SIZING BY HYDRODYNAMIC CHROMATOGRAPHY

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SUMMARY

It has been shown previously that a suspension of ultrafine particles undergoes size segregation when passed through a tightly packed column. The same effect is found for larger particle sizes in a long section of capillary tubing. The degree of particle size separation depends upon the size of the particles, the diameter of the tubing, flow rate, viscosity in the eluting phase, and the tube length. The underlying mechanism is discussed on the basis of the Segre - Silberberg tubular pinch effect. The radial particle positions in the tube as calculated from the data correspond well to the radial positions obtained in the tubular pinch effect. Possible applications and problems of using this chromatographic technique for obtaining size information on unknown particle samples are discussed.

CHAPTER I

INTRODUCTION

Many methods for separating a mixture into its components, both on the molecular and microscopic level, have been devised over the years. The basic technique to which this effort relates was first utilized by an American, David Talbot Day (7, 8), in separating hydrocarbons from petroleum, but credit for its recognition as a new process is generally given to the Russian, Mikhail Tswett (42), who separated green leaf pigments by eluting them through a tightly packed column of chalk. Different color bands corresponding to different pigments were observed moving through the column. Tswett designated the process "chromatography" because of the colors displayed. Modern techniques still bear the name although they no longer necessarily involve color.

As presently practiced, liquid chromatography utilizes a column that is typically 50 to 100 times as long as its diameter packed with (a) nonporous or porous powders which may have a liquid immobilized on their surface, (b) powders of organic or inorganic ion-exchange resins, or (c) a highly porous nonionic gel. Solvent containing dissolved components is injected at the head of the column and washed through with other pure solvent. With porous or nonporous powders, the dissolved components pass through the column in reverse order of their rates of adsorption and desorption on the packing surface or as a re-

sult of their fractional distribution between the solvent and the immobilized phase on the packing. Components having ions with different affinities for the resins likewise pass through these columns in reverse response to their affinities. In both cases the dissolved components are retarded in their passage relative to the solvent with which they were introduced. A different situation prevails in gel chromatography; here the separation is according to molecular size. Small molecules can enter the polymer network but larger molecules cannot. The smaller molecules are accordingly retarded while the large ones are swept through the column. The order of emergence is large molecules first, smaller molecules next, and the injection solvent last.

The specific technique investigated here was discovered by D. M. Ball (1) upon injecting an ink containing pigment particles at the head of an empty capillary tube which replaced the usual packed column of a chromatographic apparatus. He found that the pigment particles preceded the dissolved constituents of the ink and that the particles emerged from the capillary before the solvent in which they were injected. Subsequent investigations with pollens and yeast cells revealed that when different size particles were injected simultaneously, the larger emerged first followed by the smaller in decreasing order of size. The behavior is therefore like that of gel chromatography, but the phenomenon cannot be due to the smaller particles being held back by entrapment as in the former case; there is nothing to entrap them in an open capillary. This investigation has sought to quantify the phenomenon and to offer an explanation for it.

In traditional chromatography, matter is dealt with on a molecular level. In fact, solubility of the sample in the eluting phase has sometimes been considered necessary for successful column chromatography. However, Small (39) reported achieving an effect which is also basically identical to gel permeation chromatography by passing a suspension of ultrafine particles through a column packed with solid, nonporous spheres. As in gel permeation chromatography, the larger of the injected particles emerged first. Earlier it was suggested by DiMarzio and Guttman (11, 12) that a bed of impermeable glass beads might be used as a separation column for finite size particles. Pedersen (30) observed the fractionation of proteins in such a column and suggested that separation in this system might be caused by flow, but the idea was not developed further. DiMarzio and Guttman (11, 12) went on to suggest that a separation of particles of different sizes could be obtained due to fluid flow. They attempted to show that dilute solutions of particles undergoing Brownian motion and flowing through a capillary have average velocities which depend on particle size. Small (39) demonstrated that the effect depended upon the size of the colloidal particles as well as the size of the spheres constituting the packing. This seemed to imply that the volume or diameter of the interstitual spaces in the packing played a major role in the phenomenon. If so, it would mean that a hydrodynamic mechanism was responsible, therefore the phrase "hydrodynamic chromatography" was applied.

Using the capillary model proposed by DiMarzio and Guttman (11,12) a reasonable mechanism might be proposed. They, as well as Small, attempt

to explain the mechanism on the basis of the Brownian motion of the particles. The center of the particle (assumed to be a rigid sphere) cannot get closer to the wall of the capillary than its radius. It therefore is subjected to solvent velocities some distance from the wall . Since the solvent velocity is greater the farther from the wall, larger particles will have larger average velocities than smaller ones. Pedersen (30) suggested, however, that where Brownian motion is incidental the flow mechanism might involve the tubular pinch effect.

The so-called tubular pinch effect was first described by Segre and Silberberg (35, 36, 37) who noticed that a suspension of neutrally bouyant spherical particles transported in Poiseuille flow through a tube tended to gather in an annular region concentric to the tube inlet. They showed that a rigid sphere under the same conditions is subject to radial forces which carry it to an equilibrium position at about 0.6 of the tube radii from the axis. The lateral migration effect was subsequently confirmed by others (15, 20, 29), the observations of Segre and Silberberg spawning a remarkable number and variety of related lateral migration experiments. However, most of the experimental and theoretical results were restricted to single, rigid, spherical particles freely suspended and undergoing Poiseuille flow within a circular tube of effectively infinite length. This is quite similar to the system under investigation in this thesis. If hydrodynamic chromatography is another manifestation of the tubular pinch effect, the underlying hydrodynamics can now be readily analysed. The flow parameters which affect particle size segregation could at least be experimentally

measured with high accuracy and an insight into the underlying mechanism obtained.

Therefore, it was the intent of this investigation to explore particle size segregation in a long capillary tube, to attempt to determine what flow parameters chiefly influence this hydrodynamic chromatography, and to obtain quantitative results on these parameters. Using these results, empirical relationships between various flow conditions and particle separation are to be drawn, and an attempt is to be made to describe the underlying mechanism. Finally, some ideas as to the practical application of the phenomena for particle size determination and particle size distribution evaluation are to be offered.

CHAPTER II

INSTRUMENT AND EQUIPMENT

Basically the equipment necessary for accomplishing hydrodynamic chromatography in a capillary tube is simple. In practice, reliable and reproducible results demand close control of flow rates, high pressures, and a sensitive detector. Briefly the equipment needed consists of the following: a pump that can give a constant flow rate when operating against a wide range of pressure drops, a flowmeter, a known length and diameter of capillary tubing, an injection valve for the particles, a detector of suitable sensitivity, and a strip chart recorder. The arrangement of these components is shown digrammatically in Figure 1.

It was found that these requirements were amply satisfied by a slightly modified, commercial, high pressure, liquid chromatograph. A Micromeritics Model 7000 Series High Pressure Liquid Chromatograph, loaned for purposes of this study, was employed. This device uses a fluid drive pumping system which delivers pulseless flow without a pulse dampener, and operates from an unlimited solvent, or liquid, reservoir. It functions in both constant flow and constant pressure modes. Pressure and flow sensing feedback circuits control the instruments operation. Pressure, flow, and temperature can be electronically monitored by a digital readout device. A high pressure, septum-

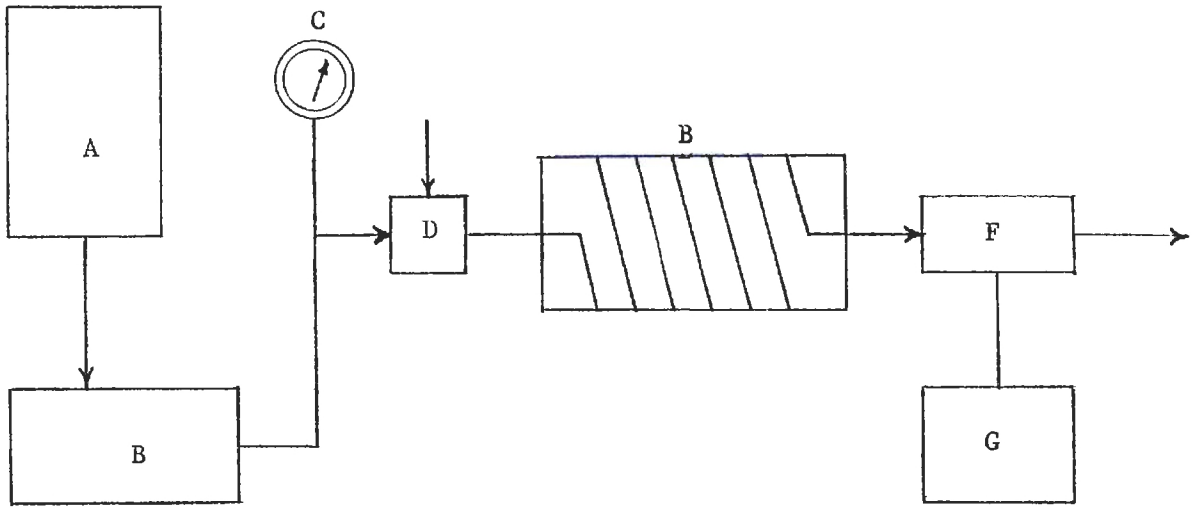

Figure 1. Schematic Diagram of Apparatus

A - Reservoir; B - Pump; C - Pressure-Flow Gauge; D - Sample Injection valve; E - Coil of Capillary Tubing; F - Detector; G - Strip Chart Recorder

less injection valve capable of injecting volumes of 1, 2, 4, or 8 microliters assured the reproducibility of injected samples, thus allowing quite reproducible data to be obtained. A flow insensitive, fixed wavelength (280 nm) ultraviolet detector with only a 10 microliter volume and a variable attenuation range is a part of the instrument. Its output was interfaced with a strip chart recorder to make a permanent record for each test.

The only major alteration made to the device was the removal of the usual column and its replacement by a know length and diameter of coiled uniform stainless steel capillary tubing. Features of the liquid chromatograph not necessary for this experiment were, of course, not utilized.

The samples employed in collecting data were principally approximately monosized sets of polystyrene and divinylbenzene spheres from the following sources having the following given diameters: Dow Diagnostics 5.7 ± 1.5 micrometers, 2.02 ± 0.0135 micrometers, 1.101 ± 0.0055 micrometers, 0.822 ± 0.0043 micrometer , 0.481 ± 0.0018 micrometer , 0.312 ± 0.0022 micrometer , 0.176 ± 0.0023 micrometer ; Dukes Standards 18 micrometers $\pm$ 1.3 percent, 9.8 micrometers $\pm$ 1.1 percent, 5.1 micrometers $\pm$ 1.2 percent. In addition a few pollens were used for testing: paper mulbery, 12 - 14 micrometers; ragweed, 19 - 20 micrometers; pecan, 45 - 50 micrometers; and corn, 85 - 90 micrometers. The marker for the bulk fluid was a 1 percent solution of acetone. The carrier fluids were distilled deionized water, and certified A.C.S. methanol. Other particle samples taken from stocks on hand were garnet, kaolin, zinc

powder, silicon spheres, and glass beads.

A Zeiss Ortholux microscope with a built-in photographic system was used for examining isolated particle fractions. All photos made on this equipment used Kodak Plus-X Pan film.

Also to insure the homogeneity of the sample mixtures and the absence of agglomeration, an ultrasonic mixer, Sonic Dismembrator, Model 12100, Quigley-Rochester, Inc., was employed in preparing samples immediately prior to each test.

CHAPTER III

PROCEDURE

The procedures employed in this investigation were designed to maintain the actual operation as simple and direct as possible, but at the same time, to probe those flow parameters which actually affected hydrodynamic separation. It was also clearly recognized that monosized particles would enhance the resolution in these tests, so nearly uniformly sized particles were utilized as detailed previously for first testing.

The handling of each injected sample was treated in as nearly an identical manner as possible. It was decided first to test each particle size singly rather than in combination with one or more other samples. This decision was based upon the observation that whether or not the particles were injected singly or in combination had little affect upon the transit time of each particle size. Also running the particles singly eased the determination of transit time due to better resolution of the recorded peaks and expedited the preparation of samples.

The samples were prepared by suspending them in a volume of the carrier fluid to a concentration of about 1 percent by weight of solids. Each sample was next treated in an ultrasonic mixer to insure that agglomeration was at a minimum. This suspension sample was then loaded into the injection valve of the liquid chromatograph. The calibrated injection valve used in the chromatograph was set for a 2 microliter sample, since this quantity usually produced an optimum peak size on

the recorder. Once this was done, the sample was introduced by merely turning the valve. Immediately upon doing this the time was marked on the strip chart recorder. The flow in the tube was then carefully monitored and adjusted, if necessary, until a peak on the strip chart indicated that the sample had passed the ultraviolet detection unit. This completed a test, the lapsed time between injection and the recording of the peak giving the time for sample passage through the column. The process was repeated until it was obvious that the results were reproducible for this particle size and flow rate. Then a test with a different particle size at a different flow parameter was made. After a series of tests was finished, the times for each were tabulated and the next series begun.

Before each series of tests both the detector and strip chart recorder were appropriately regulated to obtain optimum peak size and resolution with the smoothest baseline. This could be done by either adjusting the optical density setting on the chromatograph or the gain on the recorder. Also at this time the pressure and temperature were monitored to insure their constancy.

The general plan of research was to interchange the size of the tubing, properties of the carrier liquid, the particle size, and the flow rate, but keeping all the parameters constant except one in any one test. For instance, for a particular size tubing and a single carrier liquid at a constant flow rate, a set of particles sizes was examined. Then for a particular size tubing and a specific carrier liquid a series of flows was used with a constant particle size. Then,

in turn, a liquid with a different viscosity was used for a constant flow rate and particle diameter in a known size tube. The same type tests were also made using different diameters and lengths of tubing. Following tests with discrete particle sizes, specific liquids, known flow rates, and size of tubing, distributions of sizes and combinations of discrete particle sizes were tested in an attempt to gain further information. A microscope having an eyepiece graticule was used to examine carrier liquid fractions as they emerged from the capillary tube for size distributions. Representative slides were photographed using a built-in photographic system and Kodak Plus-X Pan film.

CHAPTER IV

RESULTS

As noted previously particle size segregation in a long capillary tube was first noticed by Dr. D. M. Ball. In preliminary experiments he successfully separated a mixture of two different size pollens into its individual components. Upon injecting a mixture of two different pollens into a liquid chromatograph equipped with a coil of three hundred feet of 0.010 inch I. D. capillary tubing, he obtained three peaks on a strip chart recorder connected to the detection unit. The first peak was found to represent the larger pollen grains; the second, the smaller size; and the final peak was material dissolved from the pollens by the carrier fluid. These findings provided the base for further investigations here.

After initial tests with various size pollens and latices, both in mixtures and separately, it was found that the difference in particle transit time for a particular size, whether in a mixture or not, was insignificant. With this in mind, first tests were made with only one particle size at a time.

The time it took for a wide range of particle sizes--from 0.481 to 90 μm in diameter--to traverse 300 ft. of 0.010 inch I. D. stainless steel capillary tubing at a constant flow of 1.25 ml/min in a methanol carrier fluid was investigated. The dependence of particle transit

time on particle diameter was readily detected, the results being given in Table I. It is also noted that results for the 85 to 90 μm diameter corn pollen was inconclusive. This is not surprizing in view of the fact that this pollen diameter is about 35 percent of that of the tubing diameter.

Having demonstrated that the effect is valid over a wide range of particle sizes, the next step was to make tests over a wide range of flow rates using narrowly distributed particle sizes. Still using 300 ft. of coiled 0.010 inch I. D. stainless steel tubing and methanol as the carrier fluid, the same tests as in the first series of trials were made for a range of flow rates from 0.75 ml/min to 3.00 ml/min at increments of 0.25 ml/min. The particles diameters used ranged from 0.176 μm to 5.7 μm. All of them consisted of very nearly monosized latex particles. The results of these tests are presented in Table II. Also, as seen in Figures 2 and 3, these results may be plotted as the base 10 logarithm of particle diameter versus the particle transit time, and the result seems to be a straight, or perhaps slightly curved line with the slope being indicative of the particle separation. It is also to be noticed that, as flow rate decreases, the absolute slopes of the lines also tend to decrease, thus indicating better separation at lower flow rates. However, results for flows less than 1.00 ml/min proved to less reliable. Possibly this is because slight errors in flow affect very low flow rates much more noticibly. Sufficiently high pressures to pump the fluid at rates greater than 3.00 ml/min could not be produced. However, within the operational limits of the

Table I. Results of First Tests

Particle Identification	Time for Particles to Emerge(1) Test 1 (sec)	Test 2 (sec)	Test 3 (sec)
0.481 μm diam. Latex	244	245	---
0.822 μm diam. Latex	242	242	---
2.02 μm diam. Latex	223	223	---
5.70 μm diam. Latex	205	204	---
Mulberry Pollen 12-14 μm	200	195	200
Ragweed Pollen 19-20 μm	153	156	150
Pecan Pollen 45-50 μm	138	140	145
Corn Pollen 85-90 μm	Distribution Inconclusive		

(1) Using 300 ft of 0.010 in. I. D. tubing with methanol as the carrier liquid flowing at 1.25 ml/min.

Table II. Transit Time for Particles

Particle Identification	Time for Particles to Emerge at Flow Rates[1] (ml/min) of 0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	3.00[2]
	(sec)	(sec)	(sec)	(sec)	(sec)	(sec)	(sec)	(sec)	(sec)
0.176μm diam. Latex	400	314	255	213	180	157	138	125	108
	405	309	257	207	177	155	138	125	108
0.312μm diam. Latex	400	304	247	204	176	153	138	122	106
	410	306	248	205	176	154	136	123	106
0.481μm diam. Latex	405	306	265	212	171	150	132	119	103
	399	308	263	206	172	150	132	121	103
0.822μm diam. Lates	396	288	237	198	170	149	132	118	103
	396	291	238	197	168	149	132	119	104
1.101μm diam. Latex	390	290	243	220	164	145	131	118	102
	380	276	236	216	165	144	130	119	101
2.020μm diam. Latex	364	306	231	198	158	140	125	112	97
	366	284	225	198	158	140	124	112	97
5.70μm diam. Latex	366	300	201	192	152	134	122	108	96
	360	285	201	195	151	133	123	109	96

(1) Using 300 ft of 0.010 inch I. D. tubing with methanol as the carrier liquid.

(2) Reynolds Numbers of 80 to 325.

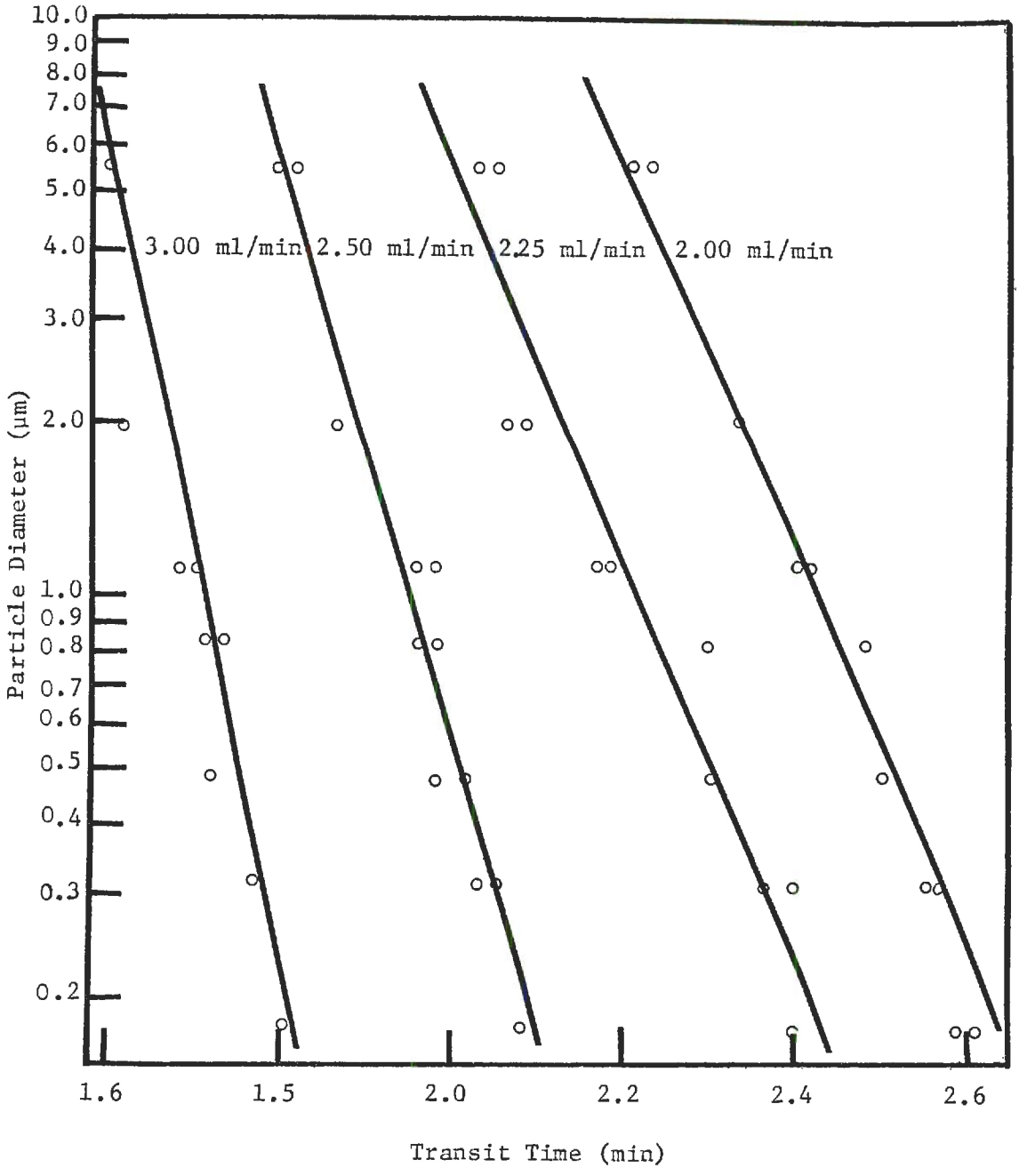

Figure 2. Particle Diameter Versus Transit Time

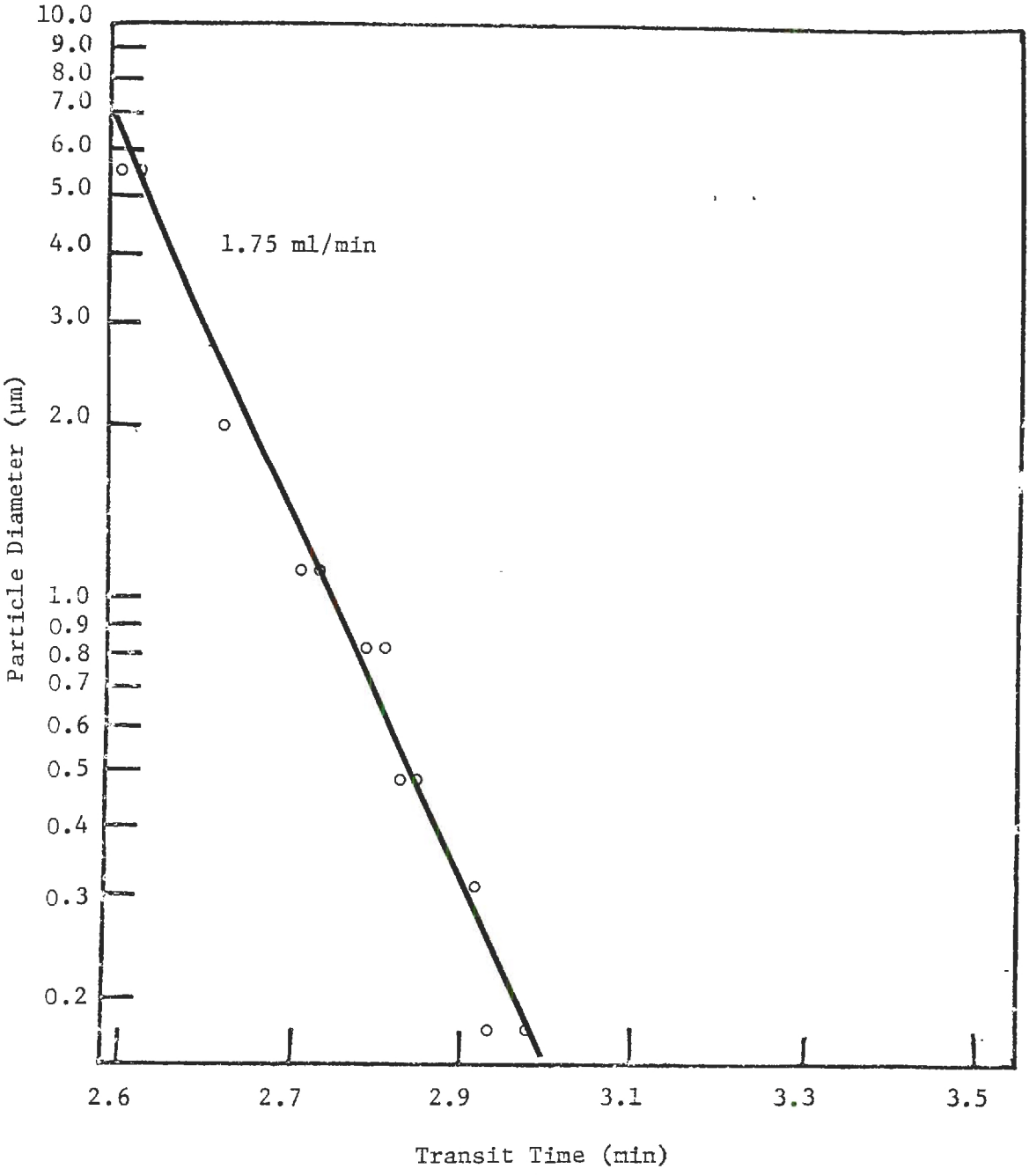

Figure 3. Particle Diameter Versus Transit Time

system, the results are reasonable and systematic. A plot of the slope of the logarithm of particle diameter - transit time data versus flow rate gives an essentially straight line as shown in Figure 4.

Also if the flow rate is plotted versus the transit time for a constant particle diameter the curve in Figure 5 results. If this is replotted as flow rate versus the reciprocal of transit time the linear plot of Figure 6 results.

These plots seem to indicate that the effect is dependent upon the shape of the velocity profile for a particular flow regime. Assuming this correct, it indicates that variations of the carrier liquid velocity profile alter particle transit times. To test this further, a liquid of a greater viscosity, water, was used. As well as providing a viscosity change, the use of water combined with the previous data with methanol should indicate whether or not the effect prevails in a liquid that is denser as well as less dense than the particles.

Selecting what seemed to be the best operating conditions from previous trials, a slightly wider particle diameter range, from 0.178 to 18 μm, was tested. First, the methanol was tested again, and then water was used. The flow rates chosen were 1.50 ml/min, 1.75 ml/min, 2.05 ml/min, and 2.25 ml/min. As before 300 ft. of coiled 0.010 inch I. D. stainless steel tubing was employed. As can be seen in Figures 7 and 8 differences between the tests with water and methanol are readily evident. One very interesting feature of these plots which may be noticed in the methanol data, but is much more clearly accentuated with water, is the curvature of the log-linear plot. Also, the general

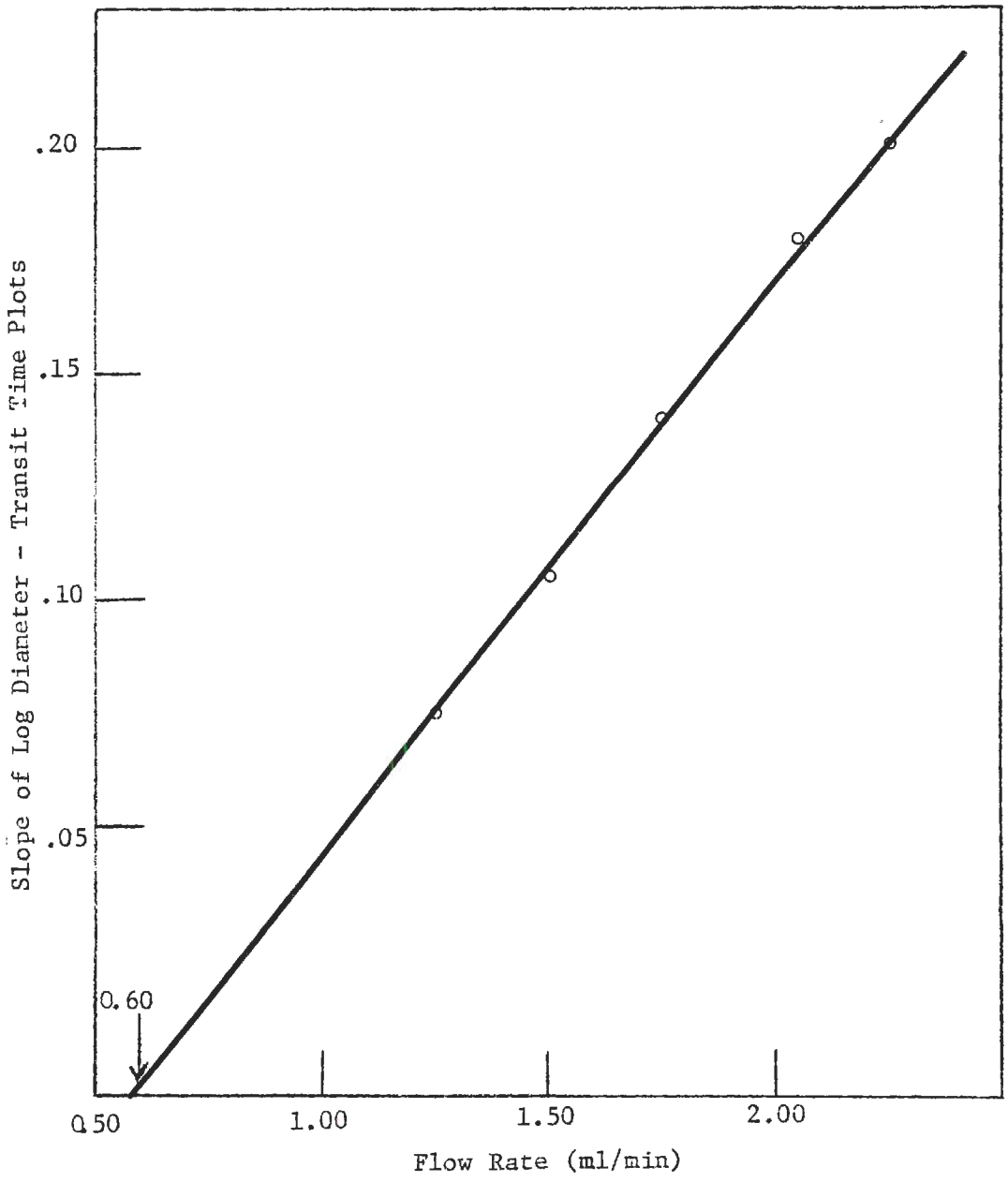

Figure 4. Slope of Log Diameter - Time Plots Versus Flow Rate

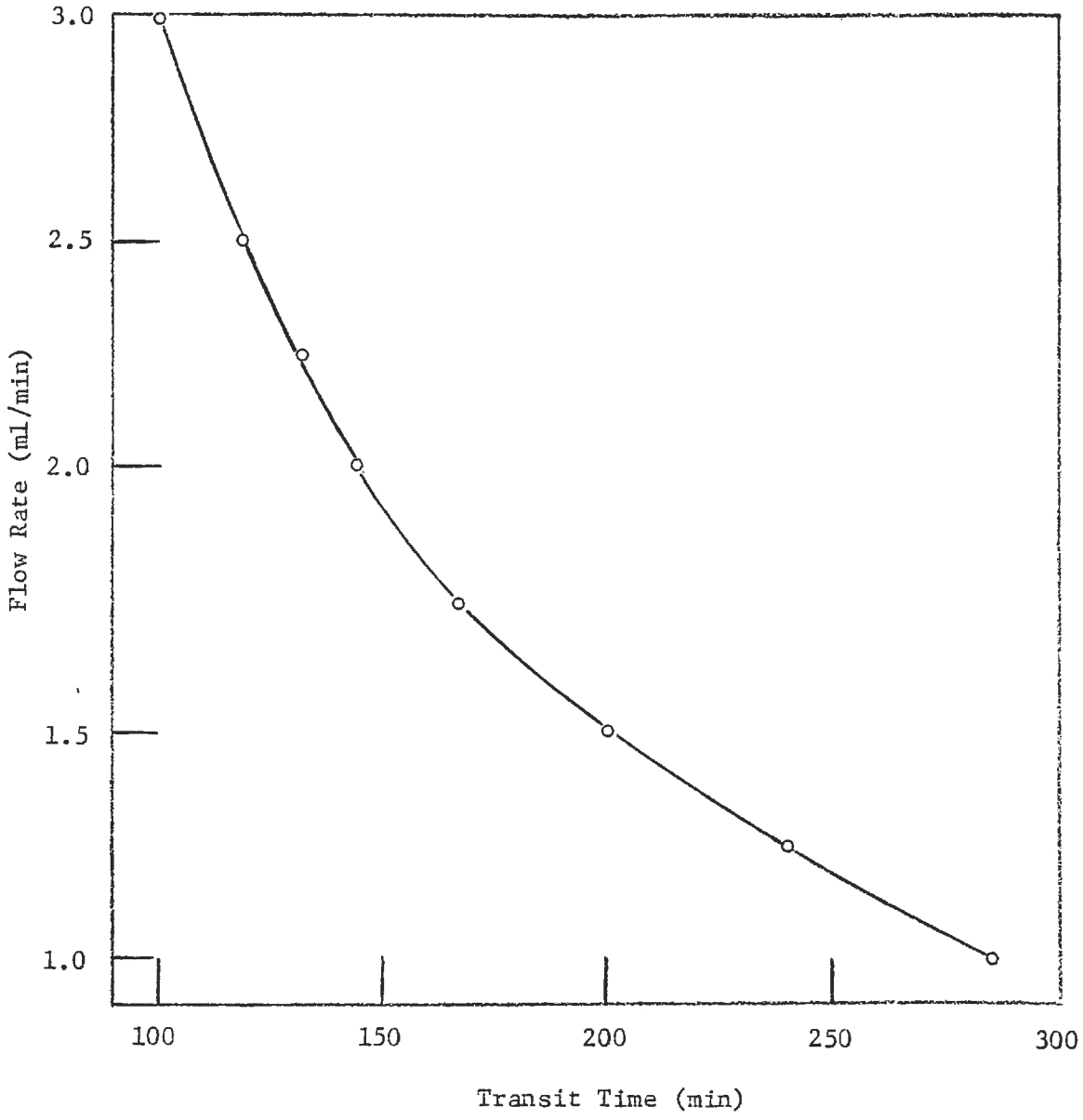

Figure 5. Flow Rate Versus Transit Time: Constant Particle Diameter - 2.02 μm

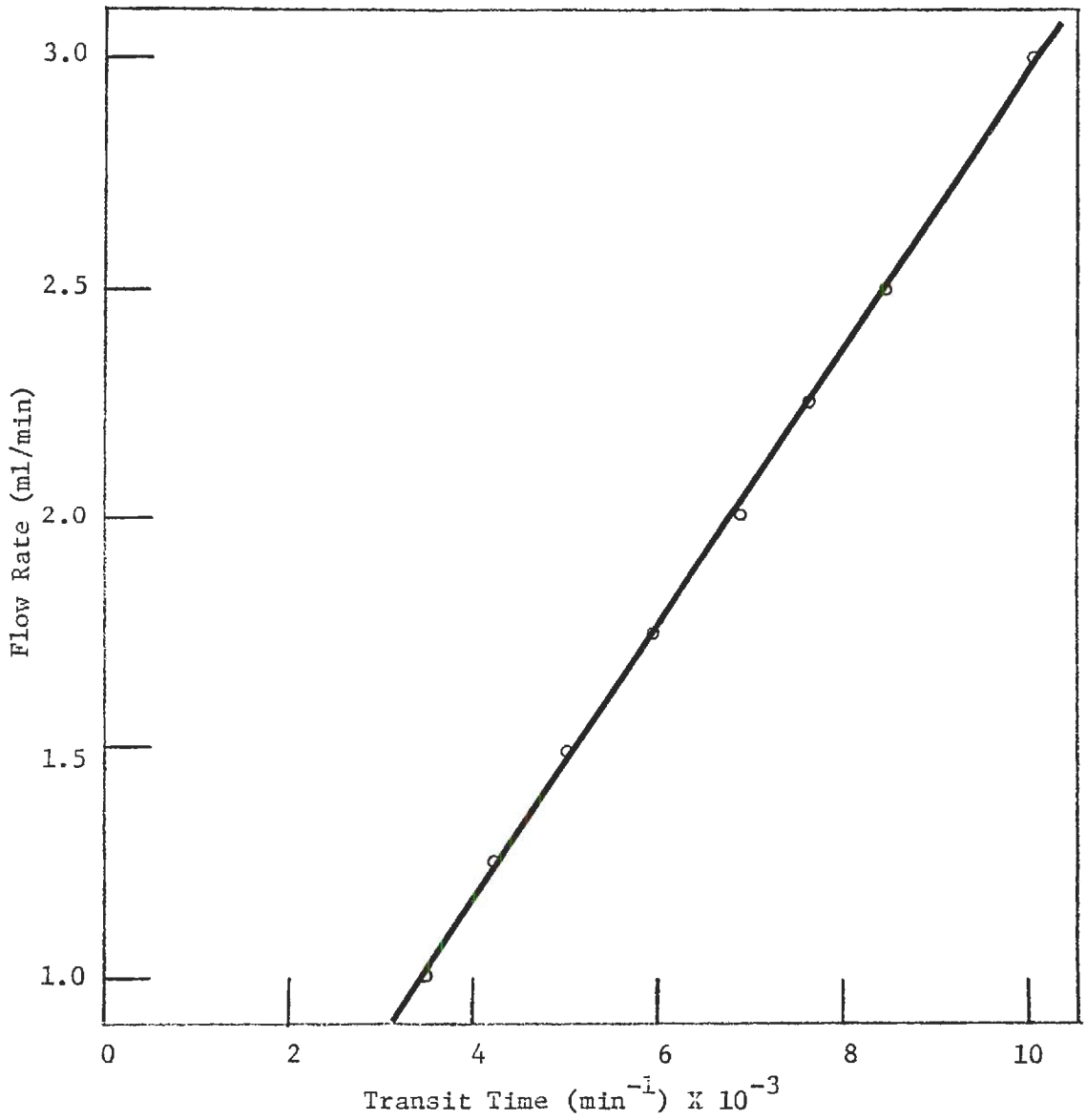

Figure 6. Flow Rate Versus Reciprocal Transit Time: Constant Particle Diameter - 2.02 μm

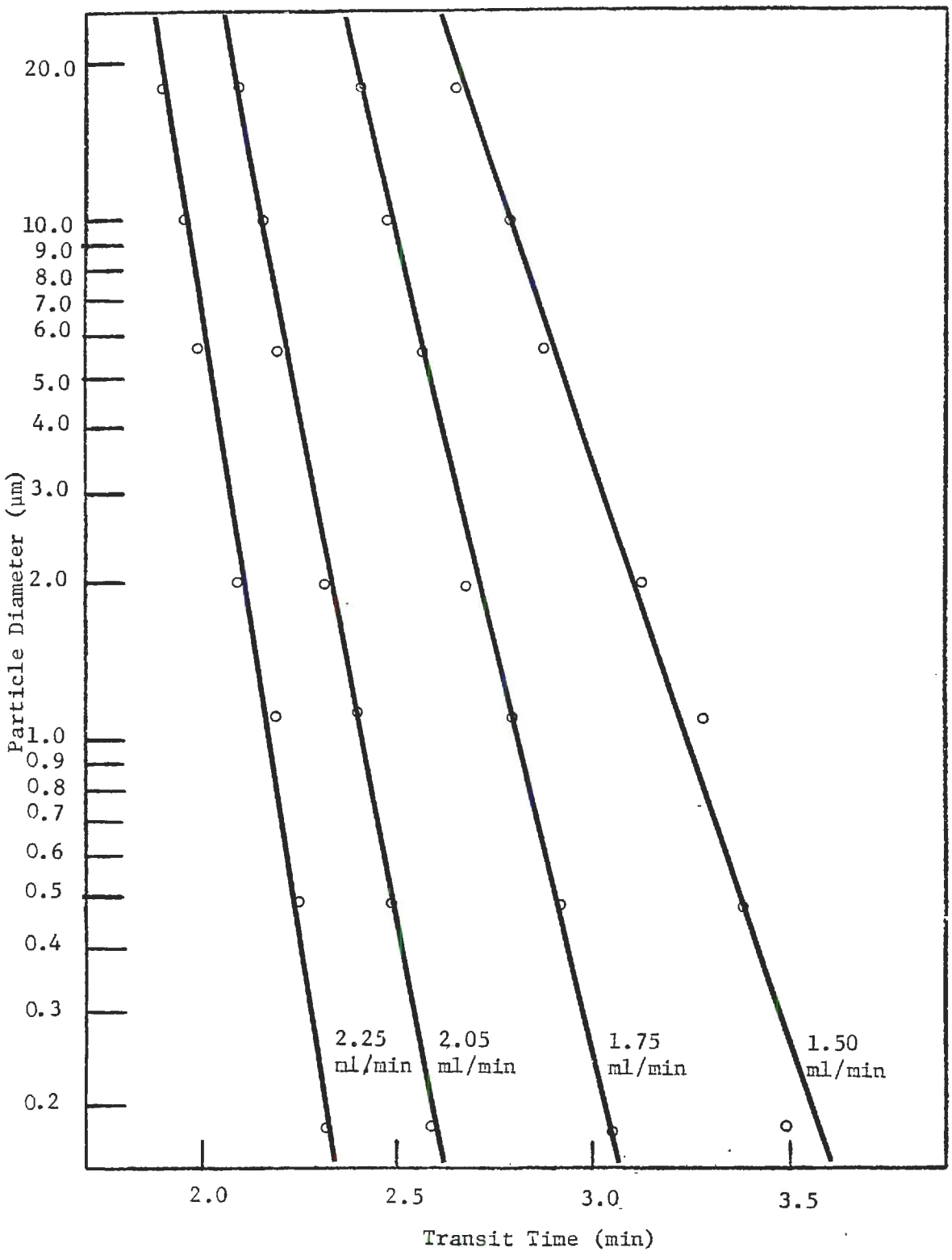

Figure 7. Particle Diameter Versus Transit Time: Carrier Liquid - Methanol

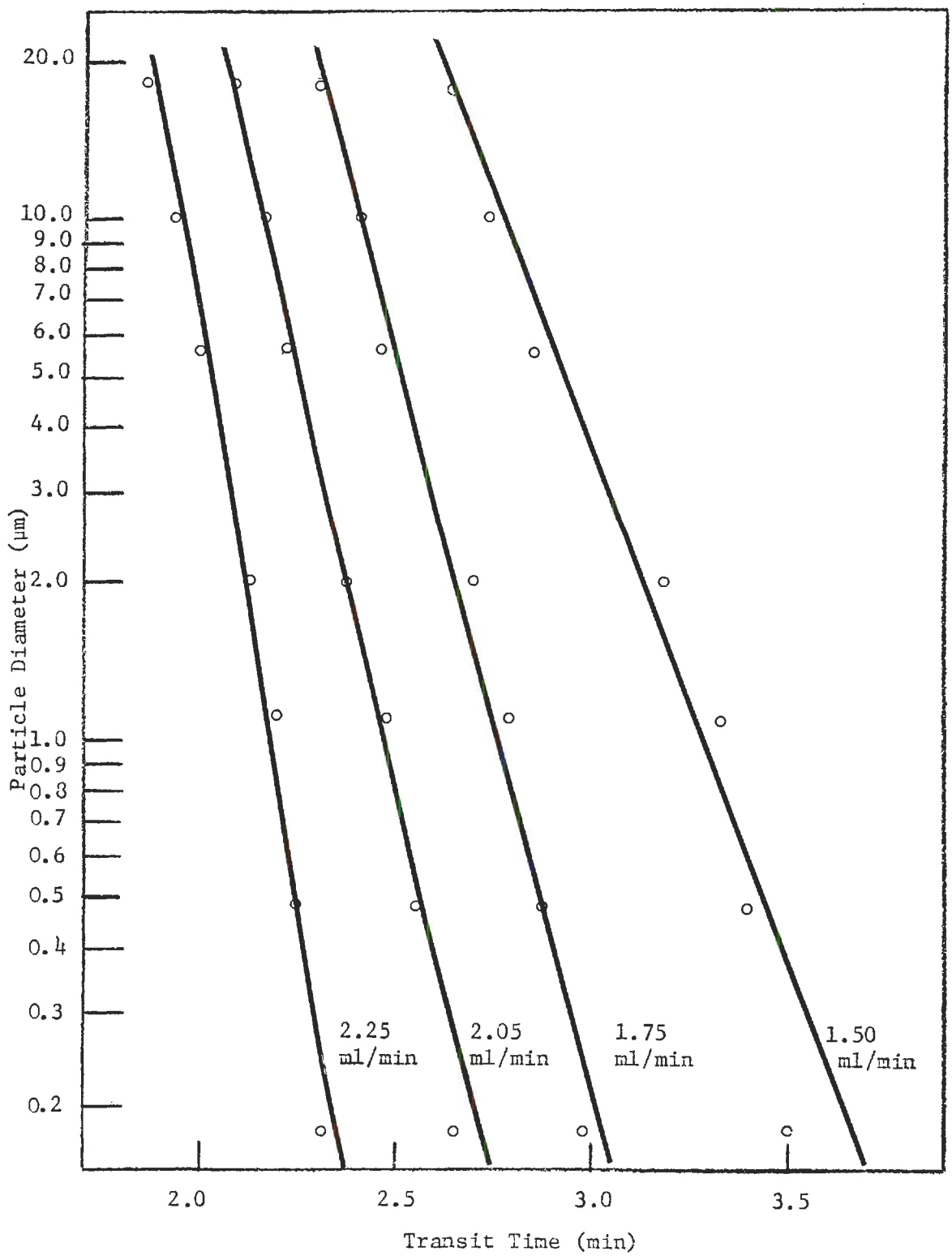

Figure 8. Particle Diameter Versus Transit Time: Carrier Liquid - Water

slope of the plots for water are not as steep as the plots for methanol. This would tend to indicate a better separation of particles with liquids of higher viscosity.

In the next series of tests the influence of tube diameter was examined. Using 300 ft. of 0.020 inch I. D. stainless steel tubing, tests comparable to those with the 0.010 inch I. D. tubing were made. When these were plotted as log-linear plots, given in Figure 9, and compared to the plots in Figure 2 a definite increase in slope can be seen. So it appears that an increase in tube diameter leads to a decrease in particle size separation. This appears once again to be directly related to the carrier liquid velocity distribution profile. Also the ratio of the particle radius to tube radius could be a factor.

It was of interest to investigate how changing the length of the tubing altered the transit time for different particle sizes, i.e., whether or not the time difference increases or decreases in proportion to tube length. Tests were made at three different flow rates using lengths of 0.020 inch I. D. stainless steel tubing of 660 and 300 ft. The results of these tests are presented in Table III. It can be seen that for approximately twice the length, twice as good separation is achieved. Therefore, it follows that separation is directly related to length.

Other tests were made to evaluate other parameters that might significantly affect hydrodymanic chromatography. It was thought that the radius of the tubing coil might affect the particle transit time. However, no significant differences in particle transit time were

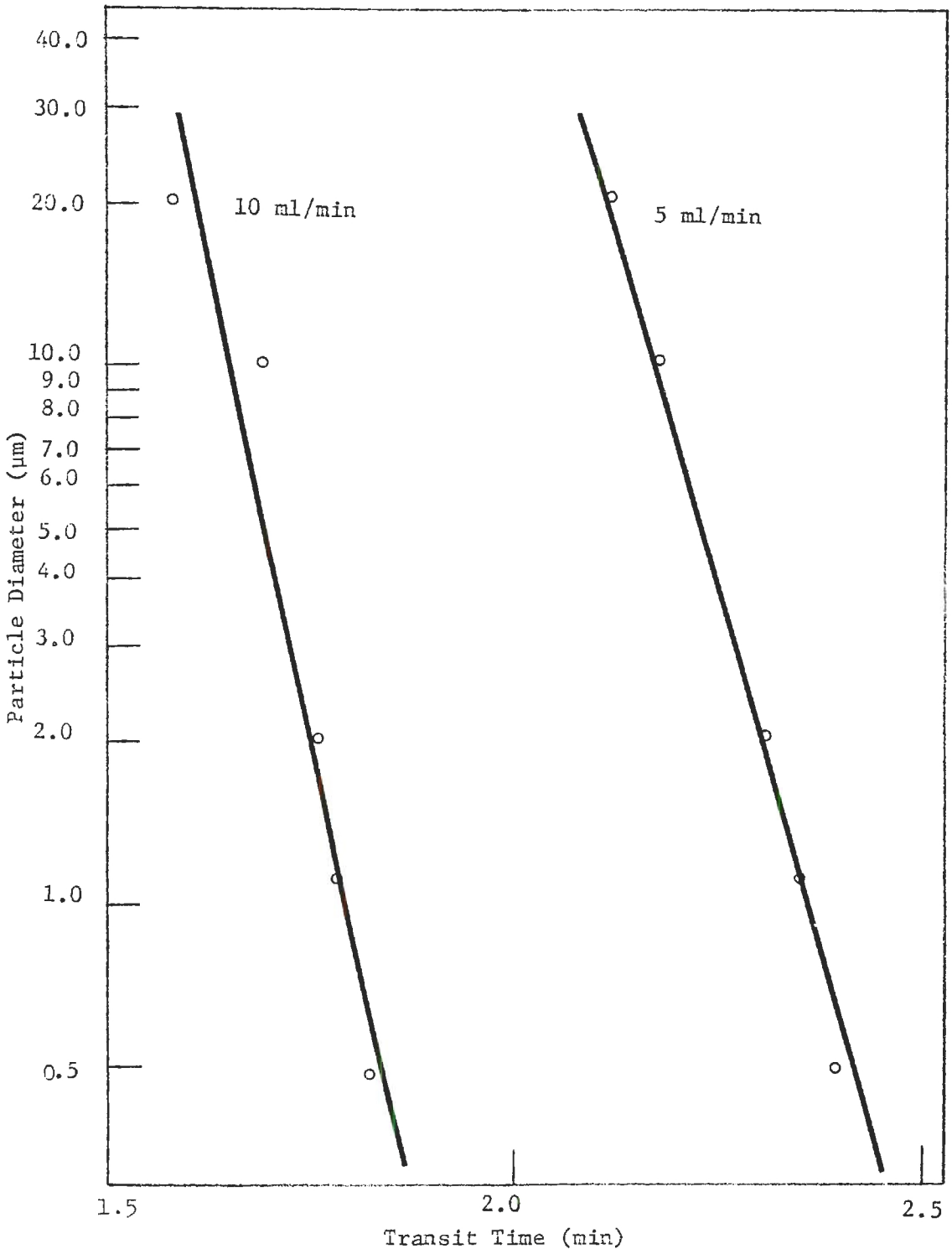

Figure 9. Particle Diameter Versus Transit Time: 0.020 inch I. D. Tubing

Table III. Effect of Tube Length Upon Size Separation for Different Flows

Liquid Flow Rate (ml/min)	Tube Length: 300 ft (1) Particle Diameter (μm)	300 ft (1) Transit Time (min)	660 ft (1) Particle Diameter (μm)	660 ft (1) Transit Time (min)
10.0 (2)	20	1.58	20	3.56
	10	1.69	10	3.96
	1.101	1.78	1.101	4.00
	0.481	1.82	0.481	4.04
7.5 (3)	20	2.12	20	5.02
	10	2.18	10	5.21
	1.101	2.35	1.010	5.38
	0.481	2.39	0.481	5.47
5.0 (4)	20	3.15	20	7.26
	10	3.23	10	7.48
	1.101	3.52	1.101	8.16
	0.481	3.58	0.481	8.17

(1) Coiled 0.020" I. D. stainless steel tubing

(2) Reynolds number of 2160

(3) Reynolds number of 1620

(4) Reynolds number of 1080

found for a radius of 6 inches and one of 2 inches. This is not to say that for an extremely small coil radius there would be no affect, but it does indicate that the radius of the tube coil is apparently insignificant when it is large compared to the inside tube radius.

Also the concentration of the particle sample was tested for effect. Concentrations of from 0.5 to 10 weight percent were tried for fixed flow rate, and no differences were found for the average transit time of the particles.

A final series of tests was made to evaluate the use of hydrodynamic chromatography for separating particles according to size and its potential use in the analysis of particles having both Gaussian and skewed distributions of sizes.

As for the separation of discreet particle sizes, this is in fact what Dr. Ball had done with the mixture of pollens in preliminary tests. As mentioned earlier, no significant difference in transit time was found for particles sizes used singly or in combination with other sizes. From Figure 10 it can be seen that there is a distinct separation of the two particle sizes, 10 and 2.02 μm, in diameter after they emerge from 300 ft. of 0.010 inch I. D. tubing. From liquid exiting corresponding to position 1 on Figure 10 the photograph of Figure 11 was taken; and finally, from the cut taken corresponding to position 3, the photograph of Figure 13 was made. It can be seen that in the central position, position 2, some overlapping of particle sizes takes place; however, on either side at position 1 and 3 the samples were distinctly separated.

With the techniques used in these experiments, resolution of

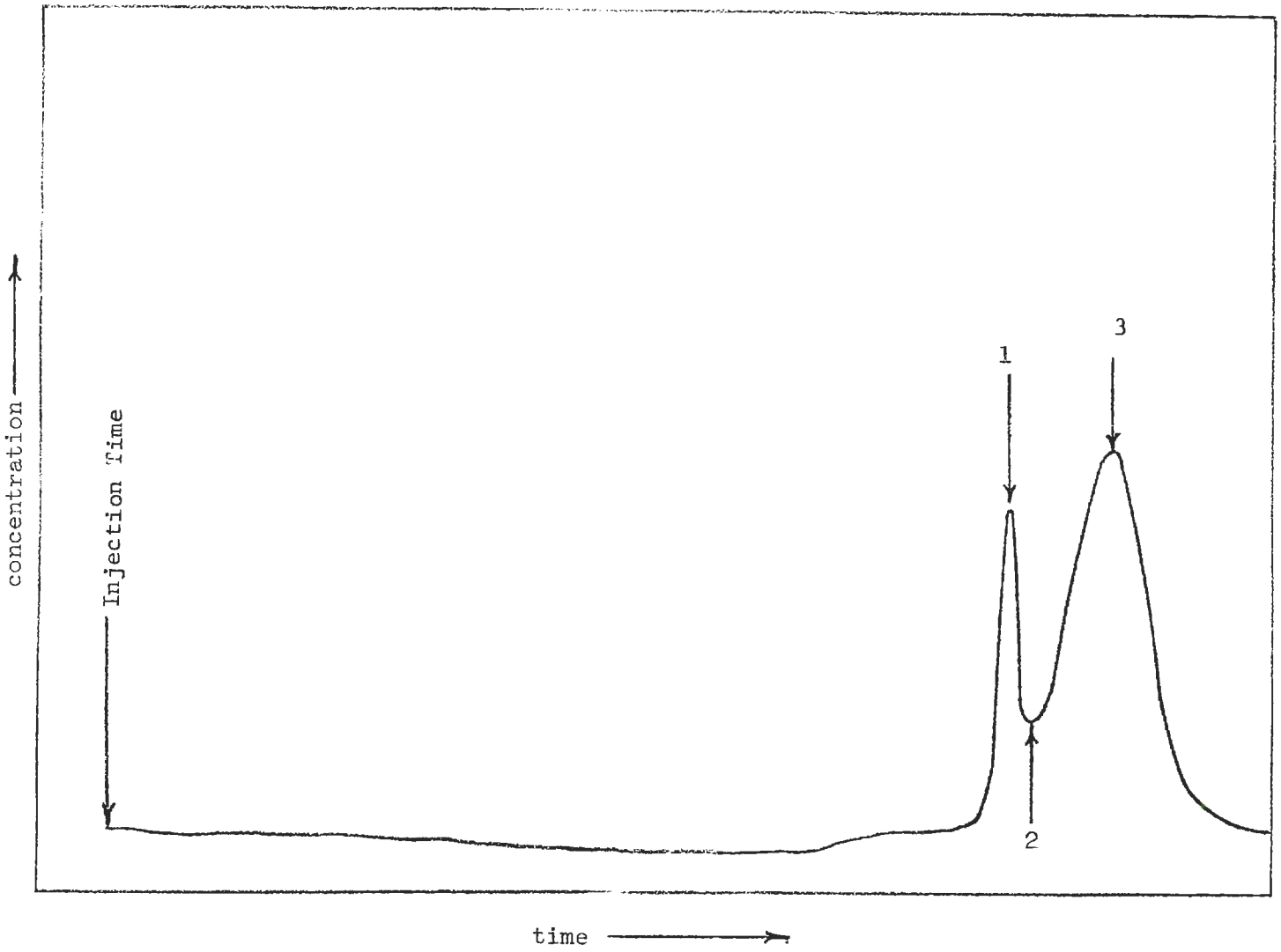

Figure 10. Particle Segregation Test

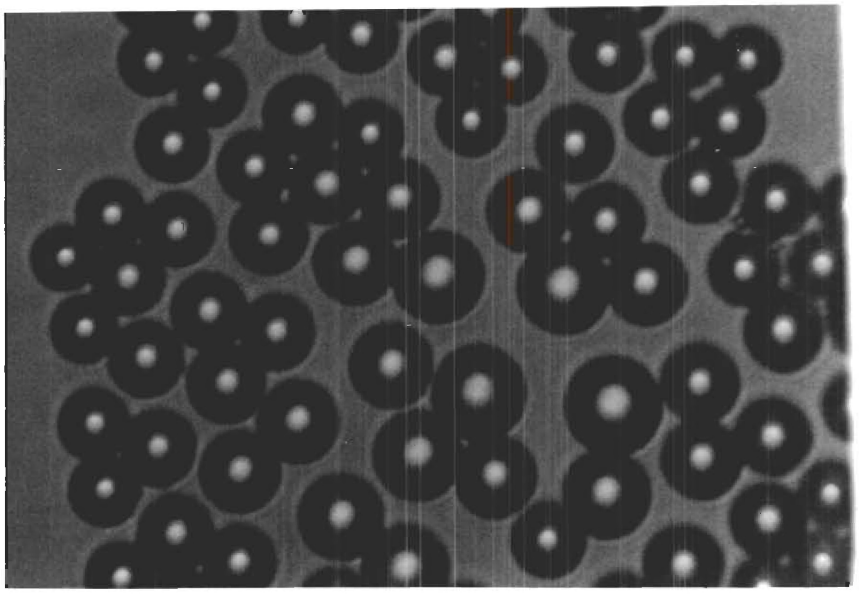

Figure 11. Photomicrograph of Fraction 1

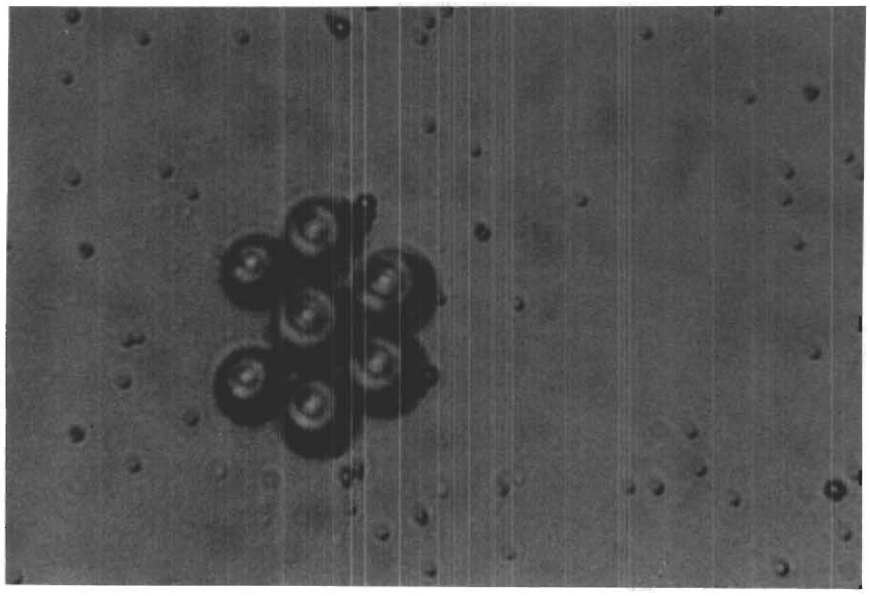

Figure 12. Photomicrograph of Fraction 2

Figure 13. Photomicrograph of Fraction 3

particle diameter differences of more than 0.2 μm in the lower size range, and 2 μm in the upper range were possible. When attempts were made to lower the flow rate to obtain better separation the peaks tended to fuse. However work done at Purdue by Dr. Fred Regnier (31), Professor of Biochemistry, with high viscosity carrier liquids, seems to indicate that much better resolution of close particle sizes is possible, especially in the extremely small sizes. In fact, macromolecules can be separated according to Prof. Regnier.

The separation seems to work quite well for any number of discreet particle sizes, as long as resolution remains good. Agglomerated particles seem to act as larger particles and exit at times appropriate for the agglomerated particle size. This was seen in tests with agglomerated pollens. However in this case, shape effects come into play and make the results difficult to analyse.

To investigate samples with particle size distributions, a sample of silicon particles with a wide,but Gaussian,distribution was injected at the head of the tubing, and appropriately, a Gaussian curve was generated with the maximum coinciding with the distribution median size. This is shown in Figure 14. Also samples with skewed distribution of particle sizes, such as garnet dust, were injected into the chromatograph. A skewed peak was the result as given by Figure 15. However, the irregular shape of the particles in this case would probably play a part in any distribution obtained. Shape effects were briefly considered, and they seemed to have a definite effect in a few preliminary tests. However, no quantitative results were obtained.

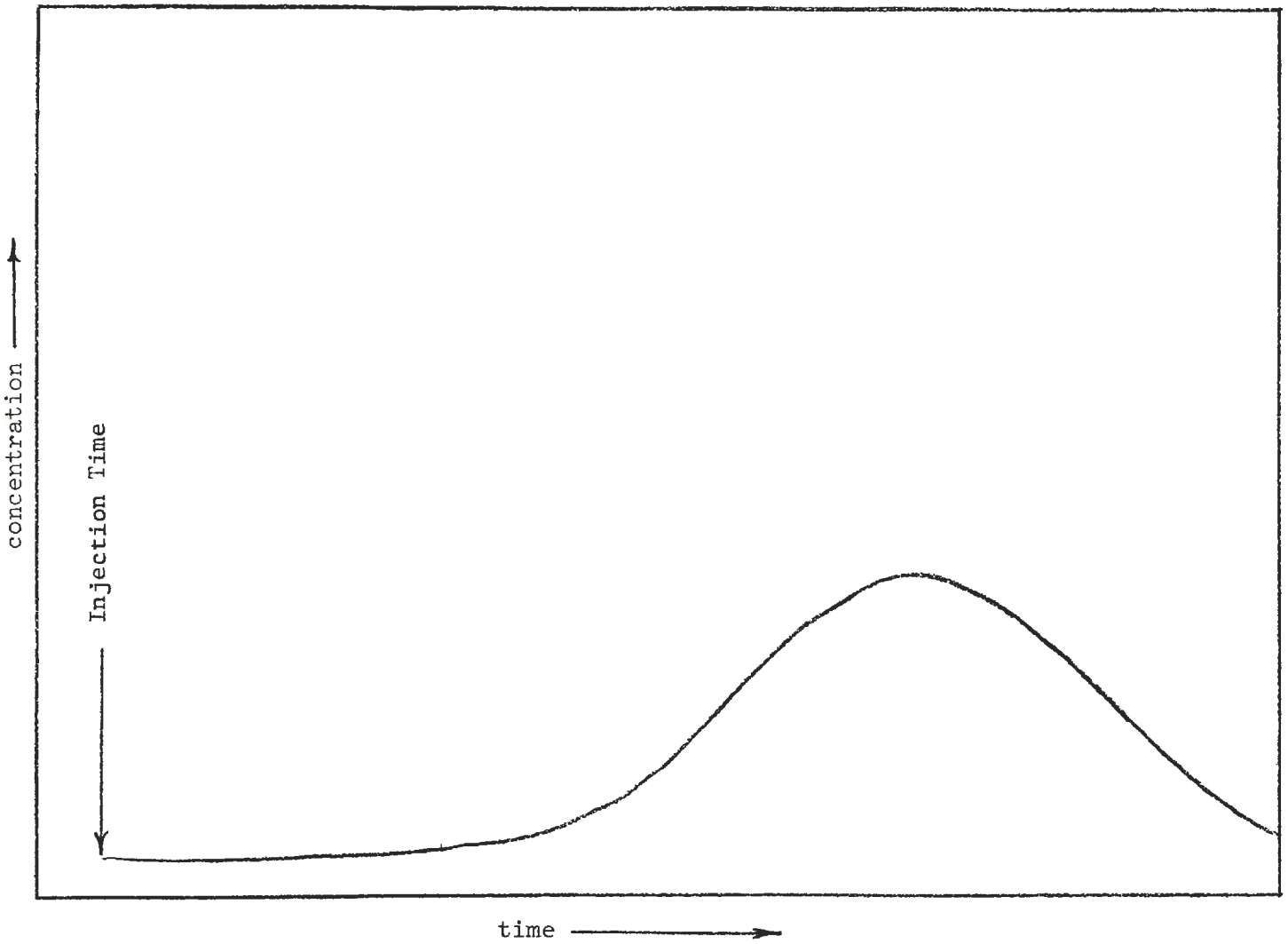

Figure 14. Gaussian Distribution Test

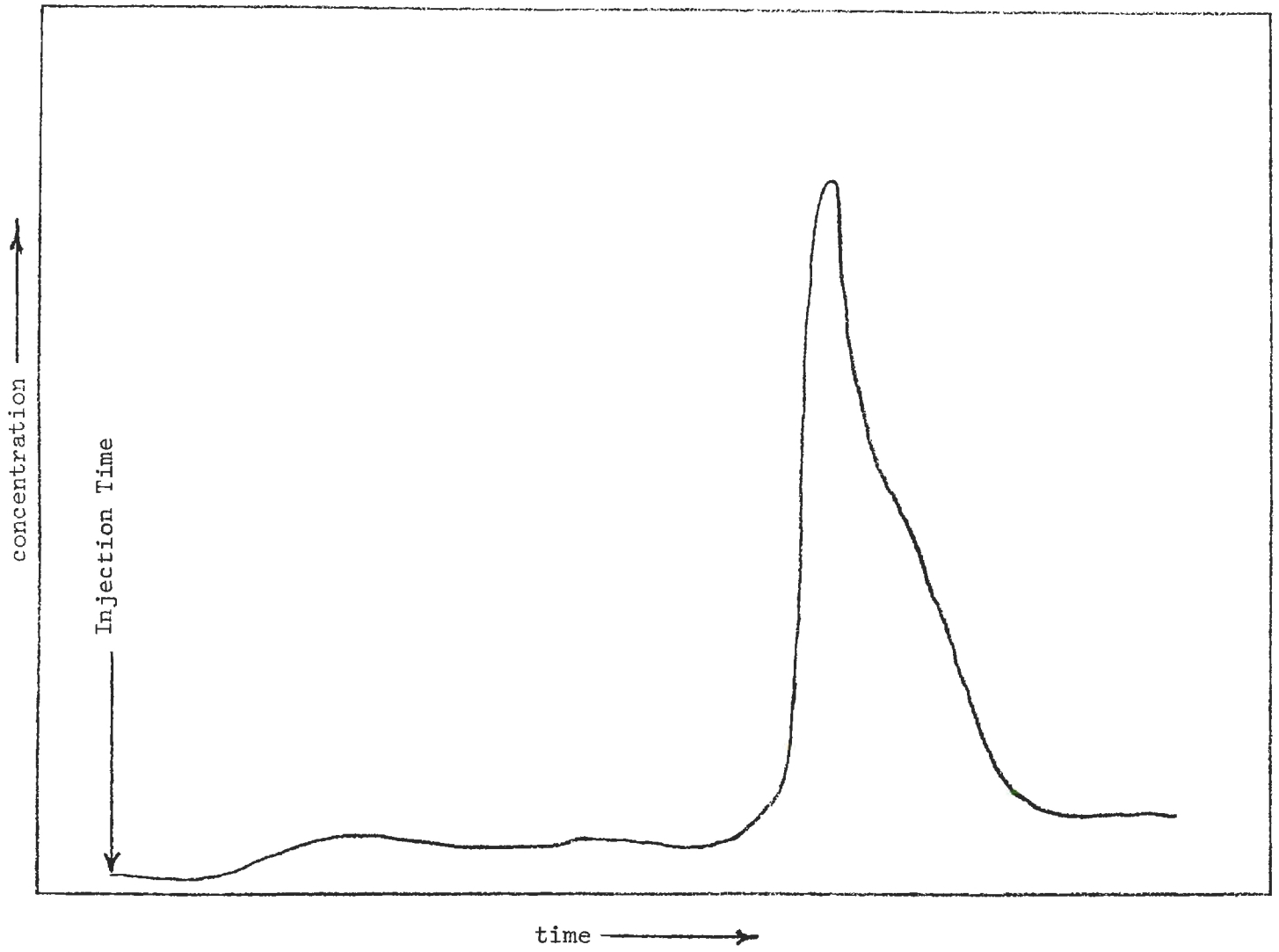

Figure 15. Skew Distribution Test

CHAPTER V

DISCUSSION

The following analysis that attempts to describe the underlying mechanism of hydrodynamic chromatography is predicated on the observation that the effect seems to have a dependency upon the liquid velocity distribution profile across the capillary tube as well as upon the size of the particles themselves. Discussed also in this section is the accuracy of the data and some of the problems with the research of this thesis. Finally, thoughts about the possible practical applications of hydrodynamic chromatography are presented.

Small (39) in his paper on the subject noted that the transit time of particles through a packed bed decreased with the diameter of the particle, and, as the size of the packing material was reduced, the particle transit time was likewise reduced. Small's proposed explanation was based upon the postulate that the interstitial sites within the packing act as capillaries. The idea was that the smaller the particles diameter, the closer the center of the particle could get to the wall. This would mean that the smaller particles could sample the very slow velocities near the wall, whereas the larger particles could not. Therefore the average velocity of the small particles would be slower than that of larger particles.

It has been noted before that the most important influence upon particle separation seemed to be the velocity profile shape within the

tube. For this to be true the particles would have to adopt the velocity of a particular position on the velocity profile. To check on this, careful examination was made of the profile and the actual times that it took for different size particles to traverse the tube. If it is assumed that the profile of velocities is described by the equation:

$$v_z = \frac{(P_o - P_L)\ R^2}{4\mu L}\left[1 - \left(\frac{r}{R}\right)^2\right]$$

where v_z is the fluid velocity at $\frac{r}{R}$, $(P_o - P_L)$ the pressure drop across the tube, L the length, and μ the viscosity, the profile for a known liquid in tubing of known size can be calculated. If then the average velocity of the known particle size is calculated from the time it takes for that specific size of particle to traverse the known length of tubing, the position for a particle traveling at that speed may be found. This has been done for methanol in 300 ft of 0.010 inch tubing in Figure 16. It is to be noted that the particles are calculated to flow in a region of from about 0.5 of the tube radii to 0.7 of the radii. As noted in Table IV this is true for a broad sample of data. Also it seems that, as shown in Figure 16, the larger the particle diameter the faster the velocity and therefore the nearer the center of the tube it seems to position itself. This implication of discreet particle positioning radially within the tube led to an investigation of the radial distribution of suspended particles in laminar flow through a tube.

Radial displacement of particles in a flowing suspension has been noted and suspected for many years. In 1836, Poiseuille noticed,

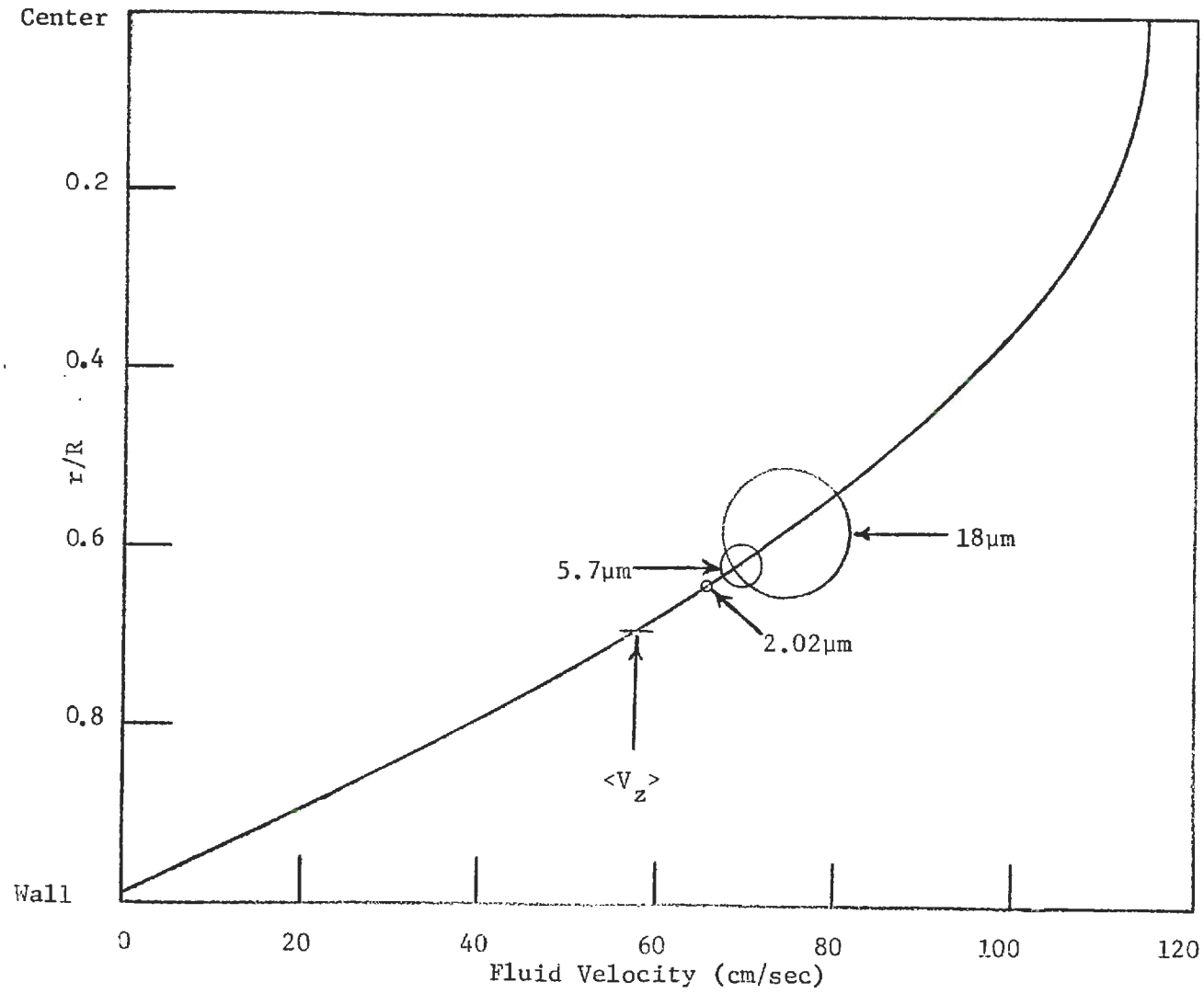

Figure 16. Velocity Profile in Capillary Tube

Table IV. Particle Positioning in the Tube as a Function of Particle Size and Flow Rate

Flow rate (ml/min)	Particle Diameter (μm)	r/R
3.00	5.7	0.690
"	1.1	0.712
"	0.178	0.731
2.05	5.7	0.615
"	1.1	0.655
"	0.178	0.685
1.25	5.7	0.607
"	1.1	0.647
"	0.178	0.683

while studying the flow of blood, that there existed a corpuscle-free region near the walls of the capillary. Along similar lines, in 1955 Taylor observed a region of low concentration not only near the wall of the capillary, but also near the center, provided the flow velocity was great enough. In 1938 Vejlons using a square-cross-section flow tube with a glass window observed that a rigid sphere released from near the wall moved away from the wall with a velocity proportional to its size.

In a more directly related finding in 1960, Segre and Silberberg found that a suspension of neutrally bouyant spherical particles when flowing through a tube of circular cross section and constant diameter became inhomogeneous in that the particles concentrated in an annular region about the tubes center. This phenomena is called the "Tubular Pinch Effect." The position at which these particles reach equilibrium is about 0.6 of the tube radii from the axis. Segre and Silberberg believed that the origin of this effect lay in the inertia of the fluid. The position is reached independent of the initial lateral location of the sphere relative to the tube axis. These findings have received support from the findings of several other researchers, notably Jeffrey and Pearson (20, 21), who made perhaps the most complete and systematic radial migration experiments yet performed. Again the effect of lateral migration was to move neutrally bouyant particles away from both the tube wall and the axis to take up instead a stable position at about 0.68 of the tube radius from the axis.

Many proposed solutions have been offered for the mechanism of

such an effect. Most experimental data obtained to date has been interpreted on the basis of the Rubinow-Keller equation for the lift force on a spinning, translating sphere in an unbound fluid at rest. In the neutrally bouyant case, this interpretation predicts a radial velocity:

$$\frac{U_L}{V_m} = -\frac{2}{9} \mathrm{Re}(a/R)^4 \beta$$

where a/R is the ratio of particle to tube radius, Re the tube Reynolds number, and β the position of the particle in the tube. This alone would act to move the particle all the way to the tube axis, which is at odds with the experimental fact that the actual stable position occurs at $\beta^* \doteq 0.5 - 0.68$. Rubinow and Keller suggest that multiplying by the factor $-(\beta^* - \beta)/\beta^*$ will bring the equation into quantitative agreement with experiment, but this suggestion has very little rational basis. In fact, a paper by Saffman (34) shows that the lift force on a freely rotating particle caused by rotation is insignificant. The data of Theodore (42) for the lift force on a nonrotating sphere in Poiseuille flow shows that lateral forces arise at small Reynolds numbers even in the absence of particle rotation. Thus, inertial lift forces can arise from "slip shear" as well as from "slip-spin".

Cox and Brenner (6) were the first to consider the complete three-dimensional Poiseuille flow problem taking account of the presence of the walls and the non-uniformity of the shear. They used the method of matched asymptotic expansions with two parameters, the Reynolds number, and the ratio a/R of the sphere-to-tube radius to solve for

the inertia-induced force and torque on the sphere. The solution is not in explicit form, but is rather a number of complex integral functions. As a result, no definite conclusions can be reached presently regarding the direction of the lateral force or its precise magnitude at a given radial position.

Showing how the tubular pinch effect relates to hydrodynamic chromatography in capillary tubing will now be attempted. If the region of the velocity distribution profile into which the particles appear to stabilize is carefully examined (Table IV) surprising similarities between this region and the positioning of particles in the tubular pinch effect may be noted. However, it must be mentioned that there are some great dissimilarities between this separation by flow and reported observations of the tubular pinch effect. Mainly, the spheres in the flow separation experiments were not neutrally bouyant. Neutral bouyancy of particles was considered a prerequisite for the tubular pinch effect in most of the experiments reported. In the tests reported here, there seemed to be little change in the effect as a result of fluid density. The latex spheres were tested in both a fluid lighter than themselves (methanol) and a heavier one (water). The results though somewhat altered, were still comparable as may be seen from Figures 6 and 7. Pedersen (30) who observed the fractionation of protein in a column packed with impermeable glass beads suggested in explanation an analogy of the tubular pinch effect. In the paper by DiMarzio and Guttman an attempt is made to explain this flow separation on the basis of Brownian motion. However, as found in this investigation, particles

too large to experience significant Brownian motion still undergo separation. This does not prove that Brownian motion plays no part in the smaller size range tested, but it cannot offer the entire explanation.

If indeed hydrodynamic chromatography in capillary tubing may be explained on the basis of the tubular pinch effect, it must first be shown that the larger particles settle into a faster velocity position than the smaller particles. The answer probably lies in the balancing of two opposing "slip" forces within the tube. The "spin-slip" force as described by the Rubinow-Keller equation forces the particles toward the axis of the tube, while the "shear-slip" force as described by Ho and Leal (19) tends to force them to migrate in the direction of increasing shear rate, i.e., towards the region near the tube wall. The magnitude of these forces would be affected by the ratio of particle-to-tube diameter. Unfortunately, an exact solution for this balance point as a function of the ratio of particle-to-tube radius does not yet exist. However, data obtained by Oliver (29) seem to indicate that larger particles do indeed tend to be closer to the tube center than smaller particles in the tubular pinch effect. In a 0.94 cm diameter glass tube, spheres of 0.23 cm diameter attained a position at about 0.6 of the tube radii from the axis and spheres of 0.30 cm at about 0.5 of the tube radii. So the idea of flow separation by the tubular pinch effect is not without basis or experimental precedent, but conclusive evidence awaits actual microscopic investigation of the particles *in situ*.

The plots of the logarithm of particle diameter *versus* particle

transit time are clearly an indication of the degree of separation of particles at a particular flow rate. As mentioned in the RESULTS section, there appears to be curvature in these plots with a slight "knee" at about one micrometer particle diameter. As to the explicit meaning of this curvature, a further investigation of hitherto unexplored parameters must be made. Although for many cases a straight line on a log-linear plot might provide a close description of the actual positioning of the particles within the parabolic flow distribution, it may not provide exact coincidence. Also it is noted that for an increased fluid viscosity, as with the water, the curvature of the plots becomes more evident. This possibly means that with viscosity change, the particle positioning in the parabolic velocity distribution coincides even less accurately with the log-linear plots.

Other forces may come into play as a function of particle size. For instance, as particle size decreases, Brownian motion becomes more and more important in defining the radial distribution. At least it might become important enough to influence or alter the tubular pinch effect separation. In this case an evaluation of wall effects as done by DiMarzio and Guttman might prove valuable. Meanwhile, evaluation of the data using a least-squares fit for the best straight line appears to be a practical approximation.

In other words, it has not yet been possible to formulate a general equation describing the situation to this time. However, this does not prevent the use of this phenomenon empirically as a particle sizing device in a thoroughly documented flow situation. For instance,

a curve for data at 1.25 ml/min using methanol in 300 ft of 0.010 inch I. D. tube could be used to evaluate an unknown particle size based upon its transit time. Although it has been shown by Goldsmith and Mason (15, 16) as well as others that the tubular pinch effect holds for particle shapes other than spheres, such as rods and discs, shape effects with present knowledge could lead to uncertainties in using this phenomena for size determination for irregularly shaped particles.

The above argument also holds for the applicability of hydrodynamic chromatography for evaluation of a particle size distribution. In addition, the radial concentration distribution of particles as a result of Brownian motion and particle size would need to be fully investigated before this method becomes feasible. Some work has already been done in this area by Segre and Silberberg (35, 36, 37). However, quite a bit remains to be accomplished before evaluation of size distributions becomes entirely practical.

Present potential of this technique is chiefly based upon the reproducibility of results. Once a calibration of a capillary tube at a known flow rate has been made, future tests at those conditions would be expected to coincide almost exactly with the calibration curve. This reproducibility permits taking spherical particles of unknown diameter and testing them at flow conditions coinciding with those of the calibration curve, and, based upon the unknown particles transit time, to determine the mean diameter of those particles with considerable precision .

Other potential uses for this effect include the separation of

the components of a mixture. As long as resolution as discussed in the RESULTS section is good, quite pure extracts from a mixture of components could be obtained as may be seen in Figures 11 and 13. This, plus information on relative particle sizes, might prove of benefit in future studies of colloidal mixtures.

CHAPTER VI

CONCLUSIONS

The conclusions that may be drawn from this work are summarized as follows:

1. Segregation according to particle size may be obtained by passing a suspension of fine particles in a liquid through a long section of capillary tubing.
2. The degree of particle size separation is directly dependent upon the size of the particles. Large particles will always precede smaller ones in the capillary to a degree dependent on the size difference between them.
3. The time-of-passage difference is related to the logarithms of the particle diameters.
4. Particle size separation by flow is favored by:
   a) lower flowrates in the capillary tube,
   b) smaller tube diameter,
   c) higher viscosity of the eluting liquid,
   d) longer length of capillary tubing.
5. The transit time for each particle size is related to the velocity profile within the liquid.
6. The radial positions the particles assume when flowing laminarly through a capillary appear to be those that would be expected if the so-called pinch effect was controlling.

CHAPTER VII

RECOMMENDATIONS

This thesis has revealed many areas for future research on hydrodynamic chromatography. The most obvious of these lies in a more exhaustive and wider ranging investigation of the parameters already discussed here. A more exact study of the dependence of separation on particle size might be accomplished using other and more nearly monodisperse particles, e.g., one-sized particles with size differences of as little as 0.1 μm. This would provide a better idea as to the exact shape of the curve of a particle diameter versus transit time plot. This in turn would provide a much better calibration curve for sizing unknown particles.

Investigation of low Reynolds numbers, possibly as low as 10 to 20 could provide interesting results. Also tests at higher Reynolds numbers than tested in this project might be of interest. This could provide a clue as to the general relationship of flow rate to particle size separation.

Changes in the viscosity of the carrier liquid seem to offer the most promise as far as increasing resolutions of particle sizes, especially for smaller size particles. A study of the effect of several high viscosity fluids might show that high particle size resolution could be obtained. Along the same lines, the effect of the particles on the local liquid viscosity might prove to be important. It has been

shown that the viscosity of actual suspensions of small particles can differ greatly and vary considerably from the predictions of the Einstein equation, for example.

The relationship of tube diameter or ratio of particle radius-to-tube radius also warrants further investigation. If the same effect may be seen for much larger tube sizes, many practical applications of the technique would probably arise. This is also true for smaller tube diameters which might allow for the separation of macromolecules.

Once sufficient results have been obtained for these parameters, a fairly accurate empirical model might be determined that could predict particle separation for any combination of flow parameters. Also, a more exact theoretical solution for the average velocity of a known particle size within the tube might be developed from what is known of the tubular pinch effect. Such a solution has been sought by many people in the past with questionable success; however, results obtained in this and future research are likely to shed new light on the subject.

In any subsequent analysis many possible factors not explored in this paper might be of importance. Particle shape would probably affect transit time through the tube and therefore particle separation. Therefore, a thorough investigation into the transit times for irregular particles should be made to indicate at least empirically the magnitude of importance of particle shape.

A more comprehensive study of the influence of particle size distribution needs to be made. Results from many types of distributions could provide a key to the effects of variations in the radial concen-

tration of each size and the part which Brownian motion plays in the phenomenon.

Accuracy in measurement of particle transit times and, perhaps, in particle concentration is a prerequisite for reproducibility of results and use of the process for practical particle size determination. Electronic timing of particle exit times to 0.1 sec would be a tremendous improvement in exact sizing, as this becomes more and more important as flow rates increase. Changes in the design of the detection unit to make it completely independent of particle size and only indicative of particle concentrations would make the analysis still more precise. This might also allow interfacing with a computer for a thorough analysis of particle sizes.

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